Pestic. Sci. 1996, **47**, 31–40

Foliar Persistence and Residual Activity of Tebufenozide Against Spruce Budworm Larvae

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(Received 30 November 1994; revised version received 5 July 1995; accepted 4 December 1995)

Abstract: A field study was conducted to investigate the persistence of tebufenozide in white spruce foliage. An aqueous suspension concentrate formulation, RH-5992 2F, was sprayed over single trees at three dosage rates, 35, 70 and 140 g of the active ingredient (AI), in 2·0 litre ha^{-1}, using ground application equipment. Foliage was collected at different intervals of time up to 64 days after treatment and tebufenozide residues were measured by high-performance liquid chromatography. Foliage was also fed to laboratory-reared 4th- and 6th-instar spruce budworm (*Choristoneura fumiferana* Clemens). The data indicated that tebufenozide residues in foliage declined with time according to first-order kinetics. The average rate-constant and half-life of disappearance (DT_{50}) were 0·0340 and 20·45 days, respectively. Larval mortality declined gradually, corresponding to the residues, but was still appreciable (49 to 70%) when the larvae were fed with foliage collected 64 days after treatment. The amount of foliage consumed by the larvae decreased when foliar residues of tebufenozide increased, thus indicating anti-feedant activity of the chemical. The LD_{50} values for both instars were similar and averaged *c.*25 ng per insect, but the LD_{90} values were significantly lower for 4th-instar than for 6th-instar, at 63·6 and 96·1 ng per insect respectively. This implies that, theoretically, at a foliar concentration of 1·0 μg tebufenozide g^{-1} foliage (fresh wt), the spruce budworm larva needs to consume 65 to 100 mg of foliage in 10 days to cause mortality in about 90% of a population of the insect.

Key words: foliar persistence, half-life of disappearance, insecticide toxicity, anti-feedant activity, tebufenozide, lethal dose.

1 INTRODUCTION

Tebufenozide, (*N-tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide; RH-5992; 'Mimic®'; Rohm and Haas Co,: Fig. 1), is a new molt-inducing insecticide introduced recently to control various lepidopteran insect pests.[1] The insecticide is a nonsteroidal ecdysone agonist mimicking the molting hormone 20-hydroxyecdysone in larval insects and exhibiting insecticidal activity by inducing premature and incomplete molting of larvae.[2] The material is toxic to lepidoptera and has a relatively low toxicity to nontarget species such as mammals, birds, fish and bees.[3] These desirable properties suggest that tebufenozide has the potential to become an effective alternative to the conventional broad-spectrum insecticides in forest insect control programs.

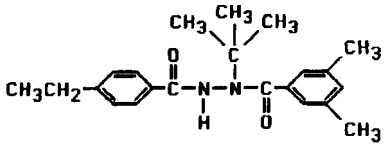

Fig. 1. Chemical structure of tebufenozide.

Literature information is sparse on the activity of tebufenozide against spruce budworm, especially after feeding on sprayed foliage that was weathered outdoors in a forest environment. Recently, several multi-disciplinary investigations were undertaken by researchers at the Forest Pest Management Institute (FPMI), Canadian Forest Service, to evaluate the effectiveness and suitability of tebufenozide to control

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Pestic. Sci. 0031-613X/96/$09.00

spruce budworm, *Choristoneura fumiferana* (Clemens), a destructive defoliator of conifer forests in eastern North America.[4] As a part of this research, the persistence of tebufenozide in white spruce [*Picea glauca* (Moench) Voss] foliage was studied after spraying at three dosage levels over young trees (with recently flushed buds), using droplet sizes similar to those found in operational spray programs. Simultaneously, budworm larvae were fed with post-spray foliage (new growth) collected at intervals of time, to determine the activity of the residues in relation to persistence. The objectives were to determine (i) the rate of decline of tebufenozide from foliage under the actual weathering conditions of a northern Ontario forest, and the half-life (DT_{50}) of disappearance, (ii) the anti-feedant activity of the chemical on budworm larvae using the relationship between residues and foliar consumption during a ten-day bioassay period, (iii) dose intake versus mortality for the 4th and 6th instars and (iv) the LD_{10}, LD_{50} and LD_{90} values for the two instars.

2 MATERIALS AND METHODS

2.1 Site description and sample tree selection

The study was conducted in a forest area about 80 km northwest of Sault Ste. Marie (46°50′N, 84°04′W) in the Goulais River basin. Sixteen uniform-sized young white spruce trees, 2·3 to 2·5 m in height and 7 to 8 cm in diameter at breast height (DBH), were selected randomly from an area of about 0·8 ha. The trees were equally divided into four groups of four. The first three groups, labelled as T1, T2 and T3, were used for tebufenozide treatment. The last group, labelled as C, remained untreated and served as the control for use in analytical procedures and for bioassays.

2.2 Laying out samplers for deposit assessment

To determine droplet-size spectra of sprays deposited at the tree canopy level, 'Kromekote'® cards (K-card, 10 × 10 cm; Intercity Papers Ltd, Mississauga, Ont., Canada) were cut into uniform strips of 2 × 30 mm. Twelve of these strips were mounted along an aluminum wire, at the rate of four strips (90° to one another) in each of three rows 8 mm apart, to simulate a natural spruce branch tip. Forty-eight of these K-card branch tips were prepared. To assess spray mass deposit, glass-fiber filters (GFF papers; PN#66211, Gelman Sciences Inc., Rexdale, Ont., Canada) were cut into strips and mounted on an aluminum wire in the same manner as the K-card strips to provide 48 glass-fiber branch tips. A set of one K-card branch tip and one glass-fiber branch tip was mounted on a spruce branch, 15 min before spray application, at mid-crown level of each quadrant of the tree [i.e. four samples (of each type) per tree × four trees per group × three groups = 48 samplers of each type in total].

2.3 Spray application

The trees belonging to T1, T2 and T3 groups were sprayed with tebufenozide at dosage rates of 35, 70 and 140 g AI ha^{-1} respectively. Immediately prior to application, pre-measured amounts of a tebufenozide 240 g $litre^{-1}$ suspension concentrate (RH-5992 2F) were vigorously mixed in 3-litre glass bottles with calculated amounts of distilled water containing 2 g kg^{-1} tracer dye, 'Rhodamine'® WT (technical grade, DuPont, Wilmington, DE, USA) to provide the above three spray mixes. A portable shelter (heavy-duty polyethylene sheet fixed to wooden frames) with dimensions of 2·0 × 2·0 × 3·0 m high was placed around each tree to prevent spray movement into neighbouring areas during treatment. The application was done on 2 June 1992 between 0630 and 1100 h, using a hand-held, battery-powered spinning disc sprayer ('Flak'; Micron Corporation, Houston, TX 77043 USA), at a flow rate 0·54 ml min^{-1}, to give a volume rate of 2 litre ha^{-1}. The sprayer was held 15 cm above the tree, and moved to and fro over the entire surface area of the enclosure to achieve uniform coverage of the tree as far as possible. At the time of application the spruce bud caps were off and the new shoots were fully exposed. The average temperature, relative humidity (RH), wind speed and cloud cover during treatment were 11·8°C, 85%, 5·2 km h^{-1} and 1/10, respectively, and there was no rainfall.

2.4 Post-spray collection of artificial samplers and spruce shoots

Both types of artificial sampler were collected 30 min post-spray. The K-card branch tips corresponding to each tree were pooled, wrapped in aluminum foil and stored at 4°C in a desiccator. The glass-fiber branch tips were pooled similarly, placed in amber-coloured jars, sealed and stored at −20°C. Samples of Spruce shoots (1992 growth, fully developed) were taken at 2·0 h pre-spray, and at nine post-spray intervals corresponding to 1·0 h after treatment (referred to as 'zero hour'), and 4, 9, 16, 23, 31, 43, 52 and 64 days after treatment. Six shoot samples were collected from each tree from the mid-crown level to provide 24 shoots corresponding to each group of T1, T2 and T3, which received 35, 70 and 140 g AI ha^{-1}, respectively. The samples were maintained frozen (dry ice) during transportation to the residue laboratory where they were stored at −20°C. Control shoot samples were also collected following the same procedure.

2.5 Analysis of droplet stains on the K-card branch tips

The stain sizes on the K-card branch tips were analyzed under an American Optical Microscope (fitted with a fibre-optics illuminator) at magnifications of 25×, 40× and 100×. The data were recorded automatically using a Wild Leitz MM 235 Measuring Device (Wild Leitz Canada Ltd, Willowdale, Ont., Canada), and the numbers of droplets cm^{-2} (droplet density) were computed. The droplet densities on the 16 samplers (i.e. four samplers × four trees) corresponding to each dosage group were pooled into one set. Similarly the data from the two remaining dosage groups were pooled into two separate sets. Using these three sets of data, the mean ± SD of droplet density was computed (Table 1). The minimum detection limit was set at 20 μm for sizing the droplet stains. The measured stain sizes were grouped into different classes and corrected for droplet spreading using spread factor data that were determined for the three tank mixes as described by Sundaram *et al.*[5,6] The spherical droplet sizes obtained were used to calculate the droplet number and volume distribution percentages (Table 1 and Fig. 2), from which the number and volume median diameters ($D_{N.5}$ and $D_{V.5}$ respectively) were calculated.[7] Table 1 also presents the maximum and minimum diameters (D_{max} and D_{min} respectively) obtained on the K-card branch tips.

2.6 Extraction and analysis of tebufenozide in spruce shoots

Aliquots of the frozen spruce needles were thawed and divided into two parts. One part was analyzed for tebufenozide residues, and the other part was fed to laboratory-reared spruce budworm larvae for bioassay. For the bioassay, it was necessary to minimize moisture loss from foliage throughout the 10-day bioassay period (otherwise larval consumption of foliage would have been affected, which would influence mortality). Therefore, a 100-μl aliquot of D-mannitol (Aldrich Chemicals, Milwaukee, WI 53233, USA) solution in distilled water (300 g kg^{-1}) was added dropwise (using a microsyringe) to 1·0 g spruce needles, with constant mixing during addition to ensure uniform distribution of the material. After repeated trials with several sugar solutions, aqueous mannitol was chosen because it helped to maintain foliar moisture levels for at least two weeks,

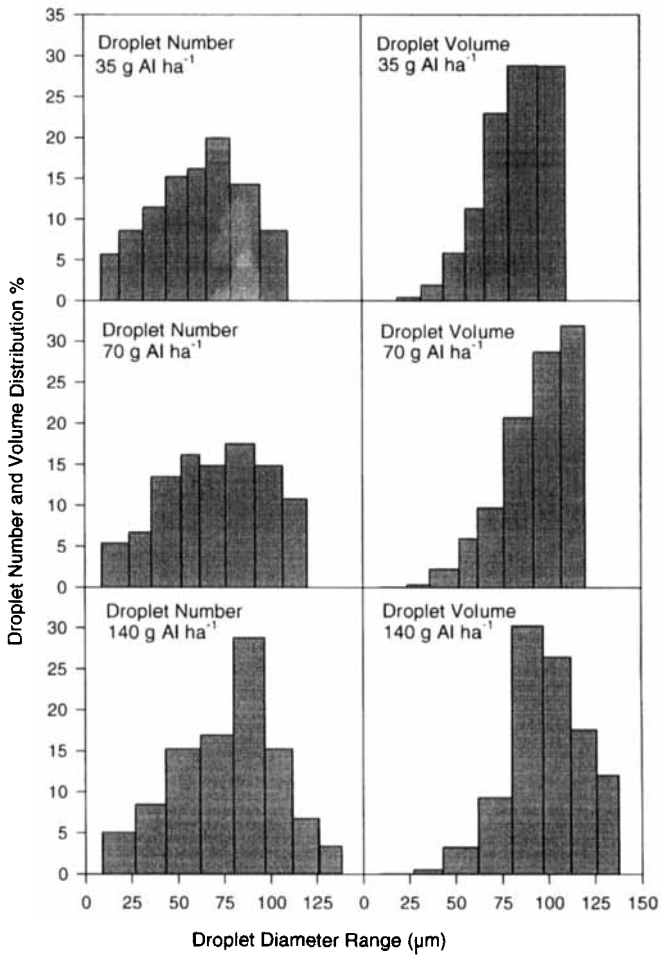

Fig. 2. Droplet number and volume distribution percentages according to size category of tebufenozide spray mixes applied at three dosage rates.

TABLE 1
Droplet and Deposit Characteristics of Tebufenozide at Spruce Canopy Level after Application of Three Dosage Rates at 2 litre ha^{-1}

Application rate (*g AI* ha^{-1})	*Droplet size spectra on K-card branch tips and tebufenozide deposits on glass-fiber branch tips* (±*SD*)					
	$D_{N.5}$ (μm)	$D_{V.5}$ (μm)	D_{max} (μm)	D_{min}[a] (μm)	*Droplets per* cm^2	*Deposit*[b] (*ng AI* cm^{-2})
35	56 (±3)	76 (±4)	110	9	105 (±11)	310 (±5)
70	64 (±4)	90 (±5)	120	9	74 (±9)	590 (±8)
140	74 (±4)	92 (±5)	138	9	59 (±10)	1260 (±16)

[a] During droplet analysis, the D_{min} of microscopic measurements was set at 20 μm for the droplet stain, corresponding to 9 μm for the spherical droplet.
[b] The deposits on the glass-fiber branch tips were extracted with a solvent and analysed by HPLC.

without interacting with tebufenozide residues, and without impairing the amount of foliage consumed daily by the larvae (see Section 2.8). To maintain uniformity of the foliar samples used for HPLC analysis and for spruce budworm bioassay, D-mannitol was added to both foliar samples.

Analytical grade tebufenozide (purity >99·6%) was provided by Rohm and Haas (Spring House, PA 19477 USA). Residues in the spruce shoots were extracted and analyzed using the high-performance liquid chromatographic (HPLC) method reported by Sundaram *et al.*[8] Briefly, the method consisted of extraction with acidified methanol solution, solvent partition, concentration and clean-up using a Florisil® column. After fractional elution of the columns with hexane + ethyl acetate as the eluent, the eluates were analyzed by HPLC using a diode-array UV detector set at 236 nm and an RP-8, 10-μm column with a mobile phase of methanol + acetonitrile + dioxane + water.

Mean recovery levels of the analyte, studied by fortifying the pre-spray and control samples of foliage (with and without D-mannitol) at 0·05 to 1·0 $\mu g\ g^{-1}$ ($n = 4$), ranged from 94 to 102% with coefficient of variation ranging from 5 to 10%. Residue data recorded in this paper include the correction for recovery efficiency. External standards were interspersed among the samples to check consistency of HPLC response. In addition, a quality control program was initiated during the course of tebufenozide analysis to monitor the accuracy and precision of the method used. Limits of detection (LOD) and limits of quantification (LOQ) of the analyte in the needle matrix were 0·02 and 0·05 $\mu g\ g^{-1}$ respectively.

None of the pre-spray or control samples of foliage contained any tebufenozide and there was no evidence of co-extracted materials causing interference with the HPLC analysis. To study the storage stability of tebufenozide, pre-spray and control needle samples (without D-mannitol) were fortified with varying levels of the analyte and stored at −20°C for 60 days. At intervals of time, aliquots were taken and analyzed using the method reported and the recoveries were quantitative. Radiotracer studies using [^{14}C] tebufenozide showed negligible adsorption of the chemical onto polyethylene, glass and 'Teflon'® surfaces.

Residues ($\mu g\ g^{-1}$ fresh wt) of tebufenozide in the spruce shoots collected at various intervals of time between 0 (1 h post-spray) and 64 days after treatment are given in Table 2. The moisture content[9] and dry weight of the samples collected at different intervals of time up to 64 days post-spray were determined, and all foliar residues were calculated based on the dry weight of shoots. However, these results were normalized according to the constant pre-spray moisture level (50·8%) of the shoots, so that the residues of AI can be expressed in $\mu g\ g^{-1}$ fresh wt.

2.7 Deposit assessment on glass-fiber filter branch tips

Spray deposits on the glass-fiber filter branch tips were extracted with acetonitrile, concentrated and analyzed for tebufenozide by HPLC without any column cleanup. The data from the 16 samplers used on the four trees corresponding to each dosage rate were pooled into one set. In this manner, three sets of data were prepared. They were converted into spray mass deposits in units of ng AI cm^{-2} (Table 1) using the surface area of the samplers and the ground area covered by the polythene enclosure around the tree.

TABLE 2
Decline of Tebufenozide Residues with Time after Spray Application on White Spruce. Tebufenozide residue ($\mu g\ g^{-1}$ fresh wt) (±SD)[a]

	Dosage (g AI ha^{-1})		
Days after spray	*35*	*70*	*140*
0	1·59 (±0·11)	3·34 (±0·09)	6·13 (±0·10)
4	1·41 (±0.11)	3·09 (±0·10)	5·98 (±0·05)
9	1·20 (±0·08)	2·70 (±0·16)	5·14 (±0.10)
16	0·95 (±0·10)	2·12 (±0·07)	4·29 (±0·07)
23	0·69 (±0·05)	1·47 (±0·08)	3·39 (±0·07)
31	0·49 (±0·02)	1·06 (±0·04)	2·08 (±0·02)
43	0·37 (±0·03)	0·74 (±0·04)	1·54 (±(0·06)
52	0·26 (±0·03)	0·54 (±0·05)	1·03 (±0·02)
64	0·21 (±0·01)	0·44 (±0·02)	0·87 (±0·01)
Rate equation	$B = 1{\cdot}615e^{-0{\cdot}035t}$	$B = 3{\cdot}490e^{-0{\cdot}035t}$	$B = 6{\cdot}560e^{-0{\cdot}032t}$
Rate constant	0·035	0·035	0·032
Coeff. deter. (R^2)	0·996	0·991	0·981
DT_{50} (d)	19·8	19·8	21·8

[a] $n = 3$.

2.8 Residual activity

The purpose of this part of the investigation was threefold: (i) to assess the anti-feedant activity of tebufenozide on the 4th- and 6th-instar spruce budworm, using only those shoots that were collected at 1·0 h post-spray (i.e. those containing initial deposits of tebufenozide); (ii) to determine the relationship between dose intake and percentage mortality for both instars using foliage collected at various time intervals; and (iii) to compute the lethal dose (i.e. the LD_{10}, LD_{50} and LD_{90}) values.

The activity of tebufenozide residues in spruce foliage was determined by means of a foliar feeding bioassay using the shoots collected at different intervals of time from the trees sprayed at 35, 70 and 140 g AI ha^{-1}. Aliquots of 1·0 g of shoots (composite sample from four trees, pooled and mixed thoroughly) were weighed into a disposable glass culture tube (13 × 100 mm) and capped with a 13-mm plastic lid containing small slits to allow air into the tube. A 100-μl aliquot of the D-mannitol solution was pipetted out, added dropwise over the shoots and mixed thoroughly as described above. For the bioassay, 4th- and 6th-instar spruce budworm larvae were collected from laboratory-reared stock.[10] The bioassay was conducted in three replicates per dosage and per sampling interval, using shoots sampled at different intervals of time up to 64 days post-spray. Shoots from the untreated trees served as the control. Fifteen larvae per replicate or 45 larvae per sample were used. One larva (previously weighed) was placed in each culture tube, and mortality was noted. The masses of the 4th- and 6th-instar larvae used were about 11 and 25 mg, respectively. The tubes containing the larvae were kept in a controlled environmental chamber at 20°C and 60% RH with a 16 : 8 h light : dark photoperiod with a light intensity of 2000 lux. The mass of each larva and the mass of foliage consumed were recorded every other day up to 10 days.

The addition of D-mannitol to foliage could influence the amount of foliage consumed by the insects, and therefore indirectly affect the mortality data. To investigate this aspect, a sample of control foliage (sample no. 1, S1) was taken every day fresh from the freezer, thawed and fed to 4th- and 6th-instar larvae for 10 consecutive days. Secondly, a large sample of the control foliage (S2) was taken from the freezer and stored in the same environmental chamber as that used for bioassay. This sample of foliage was fed to the larvae for 10 continuous days. Thirdly, a similar large sample of foliage (S3) was fortified with D-mannitol (100 μl of a 300 g kg^{-1} solution per g foliage), stored in the environmental chamber, and fed to the larvae for 10 days. In all cases, cumulative mass of foliage consumed during the 10-day period was noted. The data indicated no significant difference (Analysis of variance test[11]) between the amount consumed from S1 and S3 samples, but consumption was significantly lower (about 15%) for S2 (ANOVA $P < 0{\cdot}05$).

The mortality data from each of the three replicate samples were corrected for control mortality[12] and subjected to probit analysis[13] using POLO-PC[14] computer program to obtain linear equations for the mortality-$\log_{10}$ concentration relationships. These equations were used to determine LD_{10}, LD_{50} and LD_{90} values.

3 RESULTS AND DISCUSSION

3.1 Droplet size spectra and tebufenozide deposit

The data on droplet size spectra, droplet density and spray mass deposit, recorded in Table 1, indicate that with increasing application rate from 35 to 140 g AI ha^{-1}, the $D_{N.5}$ increased from 56 to 74 μm, and the $D_{V.5}$ from 76 to 92 μm. Analysis of variance test indicated significant difference (ANOVA $P < 0{\cdot}05$) between the three sets of data for the three dosage rates. Correspondingly, the D_{max} values also increased from 105 to 138 μm. The increase in the droplet spectra parameters of the deposit can also be seen in the histogram plot in Fig. 2. This increase could be due to the change in physicochemical properties of the three tank mixes, as a result of the increasing amounts of formulation concentrate used in the spray mix as the dosage rate increased.

The droplet density decreased progressively as the dosage rate increased (Table 1), because, for the same spray volume deposited, the larger the droplet sizes, the smaller the number of droplets per unit area. The D_{min} of the droplet stains was 20 μm (corresponding to a spherical diameter of 9 μm), because stains smaller than this value were rarely observed on the K-card branch tips. The average deposit of tebufenozide on the glass-fiber branch tips ranged from 310 to 1260 ng cm^{-2}, indicating that the deposited spray mass increased about four times corresponding to the four-fold increase in the dosage rate from 35 to 140 g AI ha^{-1}.

3.2 Rate of decline of tebufenozide from spruce shoots, and half-life (DT_{50}) of disappearance

The initial mean residue levels (μg g^{-1} fresh wt) in the shoots sprayed at 35, 70 and 140 g AI ha^{-1} were 1·59, 3·34 and 6·13 respectively. The values decreased gradually with time, probably due to climatic parameters such as light, heat and rain,[15] and oxidation, reduction and hydrolysis.[16,17] On the last day of sampling (64 days post-spray), the mean residues were 0·21, 0·44 and 0·87 for the 35, 70 and 140 g AI ha^{-1} dosage rates respectively, which corresponded to 13–14% of the initial concentrations in the needles. Thus, the rate of disappearance of tebufenozide appears to be independent of the initial deposit obtained. In general, growth

dilution and enzyme activity is high in young shoots, resulting in rapid disappearance of pesticides.[17] However, in the present study, growth dilution would not have contributed to the rapid disappearance, because the residue data, calculated on a dry-weight basis, were normalized according to the constant pre-spray moisture level. The observed long persistence (i.e. beyond 64 days post-spray) could be due to partitioning of the chemical into the cuticular waxes of spruce needles. Tebufenozide is lipophilic and has a high octanol/water partition coefficient, K_{ow} ($3{\cdot}2 \times 10^4$, log $P = 4{\cdot}5$),[1] and, therefore, it could form a solid–solid solution in cuticular waxes of the shoots, thus possibly resisting rapid loss due to environmental and metabolic factors.

The disappearance of tebufenozide in the spruce needles followed the first-order kinetics according to eqns (1) and (2)

$$B = B_0 e^{-Ct} \quad (1)$$

$$DT_{50} = (2.303 \log_{10} 2)/C \quad (2)$$

where B is the residual amount of the chemical at time t, B_0 is the initial residue, C is the rate constant and DT_{50} is the time required for 50% of the initial residues to disappear. Non-linear regression analysis of eqn (1) using the BMDP program[18] gave values of the rate constant, C, which were used to calculate DT_{50} using eqn (2). The rate equation, rate constant, C, DT_{50} and coefficient of determination, R^2, are recorded in Table 2 for each dosage rate applied.

From the data in Table 2, it is clear that tebufenozide did not disappear rapidly from the conifer needles at any of the three dosage rates. The rate constant showed a narrow range of 0·0318 to 0·0351. Similarly, the DT_{50} values also ranged only from 19·75 to 21·80 days. Statistical treatment of the data showed no significant difference in the C and DT_{50} values (ANOVA $P > 0{\cdot}05$) among the three dosage rates. The reason for this finding, in spite of the appreciable variation in initial residues from 1·59 to 6·13 $\mu g\ g^{-1}$, is not clear. Literature information on pesticide loss from environmental matrices indicates an inverse relationship between half-life of disappearance and initial concentrations.[19] The present data, however, are in disagreement with the literature findings. Further investigations are needed before a definite conclusion can be arrived at.

3.3 Anti-feedant activity of tebufenozide

The mean (±SD) of mass of untreated and treated foliage (both fortified with D-mannitol to minimize moisture loss) consumed by 4th- and 6th-instar budworm larvae during the 10-day bioassay period is recorded in Table 3. Insects that fed on shoots containing tebufenozide residues consistently consumed much lower amounts of foliage than the insects fed on untreated control foliage, and this difference was highly significant (ANOVA $P < 0{\cdot}01$). Generally, the foliar mass consumed by the larvae (both instars) decreased with increasing dosage rates, i.e. the higher the dosage rate applied, the higher the residue levels, and the lower the consumption (Table 3). In the case of 4th-instar larvae, foliar consumption at the 70 g ha^{-1} dosage rate was significantly lower than at the 35 g ha^{-1} rate (ANOVA $P < 0{\cdot}05$). At 140 g ha^{-1}, the consumption was not significantly different from the value at 70 g ha^{-1}, but was significantly lower than the value at the 35 g ha^{-1} ($P < 0{\cdot}05$). For the 6th-instar larvae, foliar consumption at the two adjacent dosage rates (i.e. at 35 and 70 g ha^{-1}, or at 70 and 140 g ha^{-1}) was not significantly different, but was significantly lower at 140 g ha^{-1} than at 35 g ha^{-1} (Table 3). Similar anti-feedant activity has been observed previously in lepidopteran insects when RH-5992 was incorporated into the diet.[2,20] At high dosage levels, this compound halted feeding and caused an ultimately lethal and developmentally premature molt of the insects.

TABLE 3
Foliar Mass[a] Consumed by Budworm Larvae in a 10-Day Bioassay Period, After Feeding on Shoots Sprayed with Tebufenozide

Application rate (*g AI* ha^{-1})	*Foliage consumed (mg) (±SD) (range)*[b]	
	4th instar	*6th instar*
0[c]	141·3[p] (±17·2) (129·7–148·8)	352·1[p] (±73·4) (334·8–365·3)
35	77·0[q] (±9·7) (61·1–95·3)	73·7[q] (±9·8) (67·2–98·4)
70	45·4[r] (±9·2) (40·1–50·9)	69·4[qr] (±10·3) (47·2–84·4)
140	39·7[r] (±8·7) (33·7–51·9)	52·5[r] (±8.7) (41·1–59·3)

[a] Foliage consumed was determined on alternate days up to 10 days after the bioassay was started.
[b] The range is given in parenthesis; $n = 45$. Values with the same superscript letters in each column are not significantly different from one another (ANOVA $P > 0{\cdot}05$).
[c] Control.

Large differences were observed in foliar consumption of the control group of larvae in the 4th- and 6th-instar stages, i.e. the 6th instar consumed on average about 2·5 times more foliage than the 4th instar (Table 3), obviously because of the greater body weight of the former. However, such a large difference in foliar consumption was not noted in insects fed on tebufenozide-treated foliage. For example, at 35 g ha^{-1}, the amount of foliage consumed was similar, 77 and 74 mg respectively, for the 4th and 6th instars (Table 3). However, at the higher dosage rates of 70 and 140 g ha^{-1}, 6th-instar larvae consumed slightly but significantly more (about 1·3 to 1·5 times) foliage than 4th-instar larvae. The present finding is obviously due to the toxic effect of tebufenozide leading to feeding reduction, and the amount consumed would be expected to vary depending on the severity of the toxic effect on the two instars.

3.4 Tebufenozide intake and budworm mortality

The percentage mortality of budworm larvae observed at intervals of time after feeding on the sprayed foliage corresponded to the residue levels in shoots, i.e. the dose (ng $insect^{-1}$) of tebufenozide ingested by the insect. As the residue (or dose) levels decreased with time, the percentage mortality observed also decreased (Table 4). However, the decrease in percentage mortality varied slightly with the dosage rate (g AI ha^{-1}) sprayed, and also with the instar of insects. In view of the several variables encountered, i.e. insect mortality, dose intake, time interval of foliar sampling, etc., it was necessary to normalize the mortality data for unit intake of dose before any realistic comparison could be made between the three dosage rates applied, or between the two instars. Accordingly, the data were normalized for unit dose, and subjected to statistical analysis [Student–Newman–Keuls test, (S–N–K)].[21] No significant difference was noted between percentage mortalities at the three dosage rates [S–N–K, $\alpha > 0{\cdot}05$]. However, significant difference was noted ($\alpha < 0{\cdot}05$) between the two instars, especially at high mortality levels. Therefore, the data from the various dosage groups were pooled for each instar to provide a consolidated three-dimensional plot (Fig. 3) using the three variables, i.e. mortality, dose and time interval of foliar sampling. Figure 3 clearly indicates a gradual reduction in mortality of insects fed on foliage collected after 20 days post-spray, especially when the dose intake was less than 50 ng $insect^{-1}$.

3.5 Lethal dose of tebufenozide to spruce budworm

The LD values of tebufenozide (and the range at 95% confidence intervals) to budworm larvae exposed to treated spruce shoots are presented in Table 5. In general, the variations in confidence intervals were higher for the LD_{10} and LD_{90} values than for the LD_{50} values. The mean LD_{10} values for the 4th instar ranged from 5·1 to 14·3 ng per insect at the three dosage rates, and the corresponding range for the LD_{90} was 48·9 to 82·5. Similarly, the mean LD_{10} values for the 6th instar ranged from 4·3 to 11·7 ng per insect, and for the LD_{90} from 89·0 to 105·5. In contrast, the variation in the individual LD_{50} values for each instar was minimal. For example, for the 4th instar, the LD_{50} values ranged only from 19·3 to 26·4 ng and for the 6th instar, the variation was slightly higher from 21·4 to 47·5 ng.

Statistical treatment using the POLO-PC computer program[14] showed that the LD_{10} and LD_{50} values for the 4th instar were not significantly different from those for the 6th instar (Table 5) ($P > 0{\cdot}05$). However, the LD_{90} values for the 4th instar were significantly lower than the corresponding values for the 6th-instar larvae ($P < 0{\cdot}05$). Thus, the data suggest that, to cause mortality of the two instars up to 50%, similar foliar residues may be equally effective. However, to cause 90%

TABLE 4

Mean Dose (ng per insect, $n = 45$) of Tebufenozide Ingested and Percentage Mortality[a] of the 4th- and 6th-Instar Budworm Larvae after Exposure to Sprayed Spruce Foliage with Tebufenozide

	35 g AI ha^{-1}				*70 g AI ha^{-1}*				*140 g AI ha^{-1}*			
Days after spray	*4th Instar*		*6th Instar*		*4th Instar*		*6th Instar*		*4th Instar*		*6th Instar*	
	Dose	*Mortal.*	*Dose*	*Mortal.*	*Dose*	*Mortal.*	*Dose*	*Mortal.*	*Dose*	*Mortal.*	*Dose*	*Mortal.*
0	92·61	100	108·23	100	134·09	100	157·84	100	206·48	100	251·82	100
4	96·65	100	90·43	84 (±10)	138·92	100	165·22	100	236·28	100	289·06	100
9	86·21	85 (±12)	90·06	75 (±9)	113·71	100	159·36	100	190·51	100	260·86	100
16	70·35	78 (±14)	67.88	86 (±17)	114·05	94 (±17)	128·44	100	162·59	100	267·27	100
23	48·92	72 (±10)	42.23	72 (±14)	77·33	100	83·52	88 (±12)	182·68	100	187·07	95 (±28)
31	38·42	74 (±10)	32·73	66 (±17)	48·21	78 (±15)	78·39	76 (±15)	88·90	100	112·01	96 (±27)
43	31·61	62 (±13)	25·83	52 (±10)	30·92	67 (±16)	62·88	63 (±14)	53·64	91 (±19)	84·84	88 (±25)
52	24·68	65 (±11)	23·03	55 (±13)	22·53	60 (±11)	45·56	56 (±15)	39·45	81 (±16)	52·43	78 (±17)
64	19·26	49 (±13)	20·96	49 (±12)	20·15	55 (±9)	45·73	49 (±7)	33·81	70 (±13)	48·03	65 (±14)

[a] Corrected for control mortality.

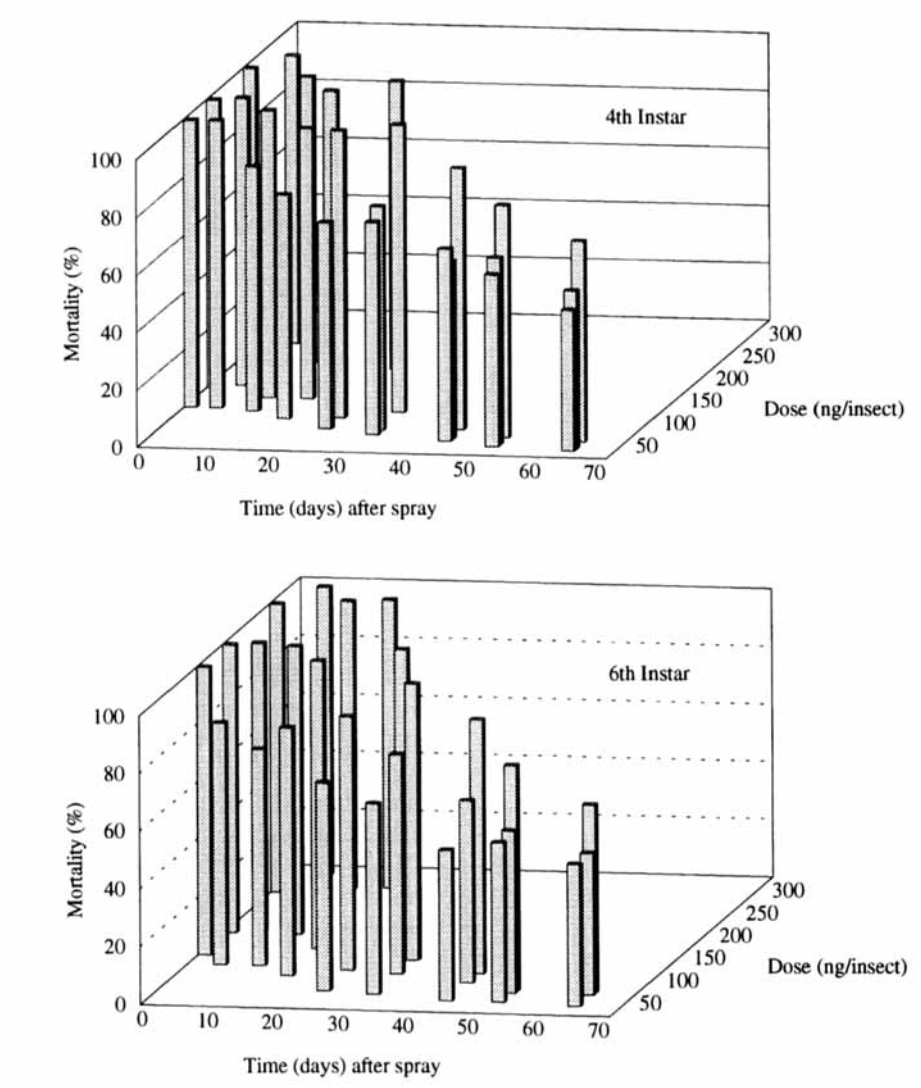

Fig. 3. A three-dimensional plot of percentage mortality versus time interval of foliage collection, and dose intake (ng per insect) for each instar.

TABLE 5

LD (ng per insect) Values of Tebufenozide to 4th- and 6th-Instar Budworm Larvae[a] after Exposure to Sprayed Spruce Needles Collected from the Field

Dosage rate applied ($g\ AI\ ha^{-1}$)	*4th Instar*							*6th Instar*						
	Slope ($\pm SD$)	LD_{10}	*95% CL*[b]	LD_{50}	*95% CL*	LD_{90}	*95% CL*	*Slope* ($\pm SD$)	LD_{10}	*95% CL*	LD_{50}	*95% CL*	LD_{90}	*95% CL*
35	2·11	5·1	0·50	20·4	8·5	82·5	58·8	1·85	4·3	0·60	21·4	11·0	105·5	72·2
	(±0·31)		10·8		28·7		195·2	(±0·28)		9·2		29·2		260·4
70	2·68	6·4	2·0	19·3	11·7	58·0	44·9	4·67	25·3	16·0	47·5	39·3	89·4	78·3
	(±0·32)		10·8		25·4		88·7	(±0·57)		32·3		53·8		110·6
140	4·79	14·3	4·5	26·4	16·1	48·9	43·3	2·90	11·7	4·8	32·2	21·0	89·0	75·5
	(±1·27)		20·7		31·6		63·8	(±0·47)		18·6		41·2		110·0
Consolidated	2·61	6·6	3·9	20·5	16·1	63·6	55·1	2·39	8·1	4·4	28·0	21·5	96·1	81·3
	(±0·20)		9·3		24·3		76·7	(±0·18)		12·0		33·7		120·6

[a] Average masses of 4th- and 6th-instar larvae ($n = 630$) were respectively 10·9(±1·1) mg and 24·8(±2·4) mg.

[b] The data represent the range obtained at 95% confidence level (CL).

control of the 6th instar, we may require higher foliar residues than to control 90% of the 4th instar. Thus, the data suggest that the 4th-instar spruce budworm larvae may be slightly more susceptible to tebufenozide than the 6th-instar, but without detailed investigations on differences in the mechanism of action of the chemical on the two instar stages, no definite conclusion can be drawn on the susceptibility aspects.

Because of the lack of significant differences in the mortality of insects at the three dosage rates applied, probit lines for the dose–response relationships of each instar stage were obtained using the pooled mortality data at the three dosages, and are presented in Fig. 4. While the slopes of the probit equations varied markedly for the two instars (from 2·11 to 4·79 for the 4th instar, and 1·85 to 4·67 for the 6th instar) at the three dosage rates applied (Table 5), the consolidated slope of 2·61 for the 4th instar was similar to the slope of 2·39 for the 6th instar (Table 5 and Fig. 4), thus suggesting only a slight difference in the dose–response relationships of the two larval stages of the budworm.

The LD data obtained from the pooled dose values using the three dosage rates for each of the 4th- and 6th-instar larvae, are more realistic and less heterogenous in reflecting the toxicity of the chemical to budworm. From the pooled LD_{50} data (Table 5), it appears that, to control 50% of the budworm populations regardless of the instar stage, the insect should ingest sufficient foliage to contain 20 to 30 ng of the chemical. For example, at 1·0 $\mu g\ g^{-1}$ fresh wt, a budworm larva has to consume 20 to 30 mg of foliage to cause 50% mortality. To achieve 90% control, the amount of tebufenozide ingested from the needles was 65 to 100 ng $insect^{-1}$ depending on the instar stage, and the corresponding foliar mass to be consumed at 1·0 $\mu g\ g^{-1}$ level, is 65 to 100 mg.

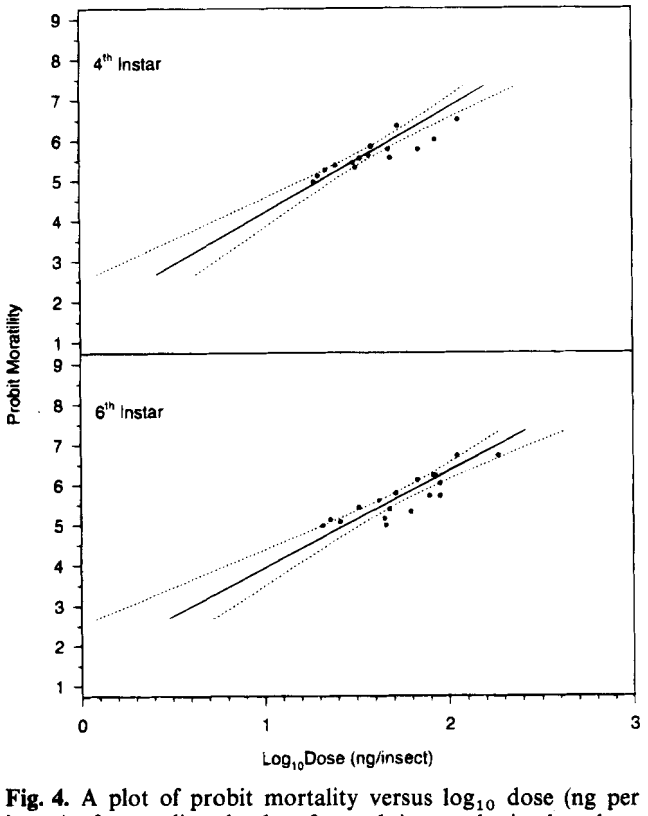

Fig. 4. A plot of probit mortality versus $\log_{10}$ dose (ng per insect), after pooling the data for each instar obtained at three rates, 35, 70 and 140 g AI ha^{-1}.

4 CONCLUSIONS

The present paper provided a novel methodology to determine the LD_{10}, LD_{50} and LD_{90} values of an insecticide that persisted for several months in the target foliage under the actual forest environment. The methodology made it possible to evaluate residual toxicity of tebufenozide to spruce budworm over a period of time after spray application. The procedure could be used on a routine basis to evaluate the toxicity of foliar residues to a wide range of insect pests, following field application of forestry insecticides.

The LD_{10} and LD_{50} values were somewhat similar for 4th- and 6th-instar larvae, but the LD_{90} values were slightly lower for the 4th instar than for the 6th instar. However, no definite conclusion can be drawn from the present study on susceptibility of the two instars to the chemical, for three reasons: (i) the role of metabolites on toxicity was not investigated in the present study, and the two instars could differ in their susceptibility to metabolites, (ii) field persistence of different metabolites could vary and provide variability in results and (iii) the mechanism of action of tebufenozide and its metabolites in budworm was not investigated in detail in the present study. Without such investigations, it would be premature to arrive at a definite conclusion on the susceptibility of the two instars.

The present study indicated that the residual toxicity of tebufenozide to spruce budworm larvae was high at all dosage rates, and the residues persisted in amounts sufficient to cause about 50% mortality even after 64 days post-spray. Ideally, an insecticide should persist in target foliage just long enough during the critical period of insect development (for spruce budworm, this period is less than a month) at a concentration above the threshold level needed to cause insect mortality, and then dissipate rapidly into innocuous products. For example, at a dosage rate of 70 g AI ha^{-1}, the residues observed in shoots were about 1·5 $\mu g\ g^{-1}$ at 23 days post-spray, which caused 100% mortality in the 4th-instar budworm, and about 90% in 6th-instar. This 23-day duration of persistence at a concentration level $\geq$ 1·5 $\mu g\ g^{-1}$ should be considered optimal for spruce budworm control. However, the slow disappearance of the chemical after the 23-day period should be viewed with caution, because of the potential toxicity to nontarget lepidoptera. The present findings suggest

the need for a thorough risk-benefit analysis before establishing the environmental acceptability of this chemical. The present findings also suggest the need for further research to determine the factors that can accelerate the rate of disappearance after the 23-day period.

It would also be difficult to extrapolate the present dose–response relationships to real-world situations. In the present study, although foliar persistence of tebufenozide was investigated under the actual field conditions, bioassays were still conducted using laboratory-reared larvae at constant temperature, RH and photoperiod. Any change in the insect colony, its ambient environmental conditions, and nature of the microhabitat, may alter the feeding behaviour and susceptibility of the insects to tebufenozide. In the real-world situation, the ambient temperature, RH and photoperiods fluctuate greatly within a day and every day, and this variation could contribute to differences in insect mobility within the microhabitat and to differences in feeding behaviour, compared to the feeding behaviour of the insects in the laboratory under constant environmental conditions.

REFERENCES

1. Anon., Data Sheet (Form 15293-4/85) on RH-5992, Rohm and Haas Company, Independence Mall West, Philadelphia, Pennsylvania, 1991, 9 pp.
2. Wing, K. D., Slawecki, R. A. & Carlson, G. R., *Science (Washington)*, **241** (1988) 470–2.
3. Burt, S. S., *Bulletin on RH-5992 Toxicology*, Rohm and Haas Company, Independence Mall West, Philadelphia, Pennsylvania, 1990, 2 pp.
4. Martineau, R., *Insects Harmful to Forest Trees*. Can. For Serv., Can. Govt Publishing Centr., Supply and Serv. Can., Ottawa, Ont., 1984, 261 pp.
5. Sundaram, A., Sundaram, K. M. S. & Leung, J. W., *Trans. ASAE*, **34** (1991) 1941–51.
6. Sundaram, A., Sundaram, K. M. S., Zhu, J. S., Nott, R., Curry, J. & Leung, J. W., *J. Environ. Sci. Health*, **B28** (1993) 243–73.
7. Johnstone, D. R., Statistical Description of Spray Drop Size for Controlled Drop Application. *Proc. Symp. Contr. Drop Appln*, British Crop Protection Council Monograph No. 22, Croydon, UK, 1978, pp. 35–42.
8. Sundaram, K. M. S., Zhu, J. & Nott, R., *J. AOAC Internat.*, **76** (1993) 668–73.
9. Anon., *AOAC Official Methods of Analysis*, 8th edn. AOAC, Washington, DC, 1955, 1008 pp.
10. Grisdale, D. G. & Wilson, G. G., *Guidelines for Rearing Spruce Budworm*. Forest Pest Manage. Inst., Canadian Forest Service, Natural Resources Canada, Sault Ste. Marie, Ontario, 1991, 13 pp.
11. Ryan, B. F., Joiner, B. L. & Ryan, T. A., *Minitab Handbook: Second Edition*. Duxbury Press, Boston, Massachusetts, USA, 1985, pp. 193–217.
12. Abbott, W. S., *J. Econ. Entomol.*, **18** (1925) 265–7.
13. Finney, D. J., *Probit Analysis*, 3rd edn. Cambridge Univ. Press, Cambridge, UK, 1971, pp. 24–33.
14. POLO-PC, *User's Guide to Probit Or LOgit Analysis*, Le-ora Software, 1119 Shattuck Ave., Berkeley, California 94707, 1987, 22 pp.
15. Sundaram, A. & Sundaram, K. M. S., *J. Environ. Sci. Health*, **B26** (1991) 115–46.
16. Van Middelem, C. H., Fate and persistence of organic pesticides in the environment. In *Organic Pesticides in the Environment*, ed. R. F. Gould. Adv. Chem. Series, Amer. Chem. Soc., Washington, DC, **60** (1966) 228–49.
17. Scheunert, I., Fate of pesticides in plants and in soil fauna. In *Terrestrial Behaviour of Pesticides*, Springer-Verlag, Berlin, **8** (1992) 77–103.
18. BMDP, *User's Digest*, 1990 Revision. Univ. Calif. Press, Berkeley, CA, USA, 1990, pp. 145–52.
19. Sundaram, K. M. S., *Pestic. Sci.*, **32** (1991) 275–93.
20. Chan, T. H., Ali, A., Britten, J. F., Thomas, A. W., Strunz, G. M. & Salonius, A., *Can. J. Chem.*, **68** (1990) 1178–81.
21. Steel, R. G. & Torrie, J. H., *Principles and Procedures of Statistical Science*. McGraw Hill, New York, NY, 1980.